Executive summary:

The relevance of neutralising antibody-mediated protection against HIV infection and disease remains to be elucidated. While antibodies are known to play an important role in protection against viral diseases such as polio, hepatitis, measles, and influenza, the relevance of neutralising antibodies (NAb) in HIV-1 protection and pathogenesis remains to be further defined. Production of an antibody response with broad neutralising activity against primary isolates of multiple HIV-1 subtypes continues to be a desired characteristic for candidate HIV vaccines. To support the evaluation of phase I, II, and III human vaccine trials testing new HIV immunogens, it will be important to standardise as far as possible and practicable and apply high throughput, sensitive, specific and reproducible HIV neutralisation assays. Numerous *in vitro* neutralisation assays have been developed, each one with different variables and endpoints.

NeutNet, started as a group of 13 participants from all over the world with the intention of co-ordinating activities aimed at standardising methods for the measurement of neutralising antibodies to HIV-1 for use in human clinical trials of candidate AIDS vaccines. The **overall goal** of NeutNet included the organization of an initial study with the most relevant neutralisation methods and a panel of well-characterised and common reagents, so as to define appropriate reference controls for neutralisation assays. The evaluation of such a study should serve as a basis for a subsequent study with polyclonal serologic reagents in order to gain an understanding of the prerequisites to accurately and reproducibly measure HIV-1 functional antibodies for HIV protection and pathogenesis. A workshop jointly organized with WHO/UNAIDS has provided a forum for the discussion of the results of the actions of NeutNet with a larger body of workers in the field and for the sharing of information at a global level.

<u>During the two-years funding NeutNet has</u>:

- enlarged the group of participants and is now composed of 18 members. Today NeutNet can provide seven different types of neutralisation assays with an array of variants in terms of 1) use of primary cells vs. cell lines, 2) use of virus isolates vs. env clones, 3) single round vs. multiple round determination, 4) timing of assay read out from as little as 2 hours to 3 - 7 days, and 5) different methods for determination of virus replication.
- organised an initial study to compare different neutralisation methods using a number of well-known monoclonal antibodies against a panel of well-characterised viruses and their envelope clones.
- defined the reagents to use for the initial study: a panel of 11 viruses covering the major subtypes and CRFs as well as phenotypes, and a panel of 4 inhibitors directed towards different epitopes of the envelope and CD4.

- prepared SOPs and the protocols to perform the initial study.
- developed and centralised the necessary and common reagents to undertake the study. Their maintenance was provided at the NIBSC repository, which has undertaken shipment of the materials to the participants.
- provided the results for data analysis and statistical comparisons.
- organised two yearly meetings to discuss the results and provided recommendations within NeutNet.
- organised a website maintained by NIBSC.
- organised a subsequent study to compare polyclonal serologic reagents such as individual and pooled sera/plasma from HIV-1-infected and uninfected donors and their immunoglobulin fraction, as well as neutralising monoclonal antibodies, in the different assays, so as to define the best conditions to determine neutralising activity.
- redefined the panel of viruses (reduced to 8 viruses) and standard inhibitors to be used in neutralisation assays.
- collected approximately 180 individual and pooled sera/plasma from HIV-1-infected individuals, which were partially tested for the capacity to cross neutralise several viruses.
- organised working groups to address specific problems of each assay type: i.e. preservation of inhibitors, production of pseudoviruses, and preparation and activation of PBMCs.
- organised a 2-days long workshop in collaboration with WHO/UNAIDS to discuss the results of actions as listed above and share the information with a larger body of workers in this field.
- disseminated the results to the global scientific community in several occasions through communication at conferences and workshops.

We are confident that the results of NeutNet will benefit HIV Vaccine research and clinical trials making it possible to compare all vaccine efforts throughout Europe and beyond.